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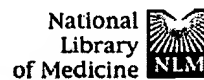
The epithelial mucin, MUC1, of milk, mammary gland and other tissues.

Patton S, Gendler SJ, Spicer AP.

Department of Neurosciences, University of California San Diego, La Jolla 92093, USA.

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MUC1 is a mucin-type glycoprotein that is integrally disposed in the apical plasma membrane of the lactating epithelial cell and protrudes from the cell surface into the alveolar lumen where milk is stored. Envelopment of milk fat globules by this membrane accomplishes their secretion and conveys MUC1 into milk. The human form of this mucin has been detected in many other organs, tissues and body fluids. It projects from the cell surface as long filaments. In the human and a number of other species, MUC1 is polymorphic due to variable numbers of a tandemly repeated segment 20 amino acids in length. The individual codominantly expresses two alleles for the mucin so that differences in its size among individuals and between the two forms of an individual are observed. The tandem repeats are rich in serines and threonines which serve as O-glycosylation sites. Carbohydrate content of MUC1, as isolated from milk of human, bovine and guinea pig, is approximately 50%. The oligosaccharides carry substantial sialic acid at their termini and this accounts for two putative functions of this mucin, i.e., to keep ducts and lumens open by creating a strong negative charge on the surface of epithelial cells which would repel opposite sides of a vessel, and to bind certain pathogenic microorganisms. MUC1 is protease resistant (trypsin, chymotrypsin and pepsin) and large fragments of it can be found in the feces of some but not all breast-fed infants. MUC1 has a highly varied structure because of its polymorphism, qualitative and quantitative variations in its glycosylation between tissues, individuals and species, and differences due to divergence in the nucleotide sequences among species. Sequencing of the MUC1 gene for various species is showing promise of revealing unique evolutionary relationships and has already indicated conserved aspects of the molecule that may be functionally important. Among these are positions of serine, threonine and proline in the tandem repeats and a high degree of homology in the transmembrane and cytoplasmic segments of the molecule.



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Early steps in the biosynthesis of MUC2 epithelial mucin in colon cancer cells.

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McGuckin MA, Devine PL, Ward BG.

Department of Obstetrics and Gynaecology, University of Queensland, Royal Brisbane Hospital, Herston, Australia.

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Expression of the MUC2 mucin has been demonstrated in normal gastrointestinal and respiratory epithelium and in carcinomas of the gastrointestinal and respiratory tracts, breast, ovary, and bladder using RNA probes and (or) monoclonal antibodies reactive with peptide epitopes on the 23 amino acid tandem repeat. Mouse monoclonal antibodies 4F1 and 3A2 were previously obtained by immunization with mucin derived from the LS174T colon cancer cell line and a KLH conjugate of a synthetic MUC2 VNTR peptide. These antibodies react with distinct epitopes on synthetic VNTR peptides and with normal and malignant epithelial tissues. In the present study, we examined the biosynthesis of MUC2 in LS174T colon cancer cells, using these antibodies to immunoprecipitate labelled mucin. A very high molecular mass protein was immunoprecipitated following 1 min pulse labelling with [3H]threonine and [3H]proline. A slight increase in molecular mass was observed over the next 16 min; however, unlike the MUC1 mucin, there was no large difference in apparent molecular mass between the MUC2 protein precursor and fully processed mucin using separation by SDS-PAGE. O-Glycosylation began within 1 h of synthesis of the protein core. Mucin secretion into the culture medium was detected in the 2nd hour following synthesis and was largely completed within 4 h of synthesis. Secreted mucin was far less reactive with these monoclonal antibodies than the precursor protein.

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Molecular cloning and analysis of the mouse homologue of the tumor-associated mucin, MUC1, reveals conservation of potential O-glycosylation sites, transmembrane, and cytoplasmic domains and a loss of minisatellite-like polymorphism.

Spicer AP, Parry G, Patton S, Gendler SJ.

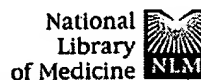
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London, United Kingdom.

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We present here the full-length cDNA sequence and genomic structure of the mouse homologue of the tumor-associated mucin, MUC1. This mucin (previously called polymorphic epithelial mucin) is present at the apical surface of most glandular epithelial cells. The mouse gene, Muc-1, encodes an integral membrane protein with 40% of its coding capacity made up of serine, threonine, and proline, a composition typical of a highly O-glycosylated protein. The mucin core protein consists of an amino-terminal signal sequence, a tandem repeat domain encoding 16 repeats of 20-21 amino acids, and unique sequence containing transmembrane and cytoplasmic domains. Homology with the human protein is only 34% in the tandem repeat domain, mainly showing conservation of serines and threonines, presumed sites of O-linked carbohydrate attachment. Homology rises to 87% in the transmembrane and cytoplasmic domains, suggesting that these regions may be functionally important. The pattern of expression of the mouse mucin is very similar to that of its human counterpart and accordingly the two promoter regions share high homology, 74%, although previously identified potential hormone-responsive elements are not conserved. Interestingly, the mouse homologue, unlike its human counterpart does not exhibit a variable number tandem repeat polymorphism. We present evidence that suggests that the mouse gene was at one time polymorphic but has mutated away from this state.

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Purification and characterization of a membrane-bound and a secreted mucin-type glycoprotein carrying the carcinoma-associated sialyl-Lea epitope on distinct core proteins.

Baekstrom D, Hansson GC, Nilsson O, Johansson C, Gendler SJ, Lindholm L.

Department of Medical Biochemistry, University of Goteborg, Sweden.

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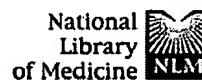
Two mucin-type glycoproteins detected by the monoclonal antibody C50, which reacts with the carcinoma-associated sialyl-Lewis a and sialyl-lactotetraose epitopes, were found in secreted and solubilized materials from the colon carcinoma cell line COLO 205. The larger glycoprotein (H-CanAg; heavy cancer antigen) was predominantly found in extracts of cells grown in vitro or as nude mice xenografts whereas the smaller species (L-CanAg; light cancer antigen) was the major component in spent culture medium and serum from grafted mice. Using detergent in the extraction buffer doubled the yield of H-CanAg, suggesting that this glycoprotein is membrane bound whereas the yield of L-CanAg was relatively unaffected. The two glycoproteins were purified from xenograft extracts and spent culture medium using perchloric acid precipitation, monoclonal antibody affinity purification, ion exchange chromatography, and gel filtration. Both glycoproteins were unaffected by reduction and alkylation in guanidine HCl. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, relative molecular masses were estimated to be 600-800 kDa for H-CanAg and 150-300 kDa for L-CanAg. Carbohydrate analysis revealed that the CanAg glycoproteins were highly glycosylated (81-89% carbohydrate by weight), carrying carbohydrate chains with average lengths of 13-18 sugars which were rich in fucose and sialic acid (2-3 residues/chain for each sugar). L-CanAg isolated from spent medium was glycosylated to a higher degree than its counterpart from xenograft extract. Immunochemical studies of the intact glycoproteins showed that both H-CanAg and L-CanAg expressed the monoclonal antibody-defined, sialic acid-containing carbohydrate epitopes CA203 and CA242 as well as the Lewis a blood group antigen whereas only H-CanAg appeared to carry the sialyl-Lewis x epitope. The amino acid compositions were typical of mucins, containing high amounts of serine, threonine (more than 25% together), and proline (11-18%). Significant differences in amino acid composition between H-CanAg and L-CanAg were found. A rabbit antiserum against the cytoplasmic C-terminal part of the MUC1

gene product, core protein of the carcinoma-associated polymorphic epithelial mucin (PEM) and DU-PAN-2, reacted with H-CanAg. After deglycosylation with trifluoromethanesulfonic acid, H-CanAg but not L-CanAg was recognized by the monoclonal antibodies SM-3 and HMFG-2, directed to the tandem repeat of the PEM apoprotein. However, these antibodies which react with PEM from mammary carcinomas without prior deglycosylation were unable to recognize intact H-CanAg, probably as a consequence of a more extensive glycosylation of this glycoprotein.(ABSTRACT TRUNCATED AT 400 WORDS)

PMID: 1718981 [PubMed - indexed for MEDLINE]



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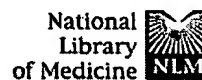
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Molecular analysis of a protein highly expressed in human breast cancer, indicates the presence of a polymorphic tandem repeat domain that encodes a conserved 20 amino acid repeat motif rich in serine and threonine residues that in the mature protein, designated MUC1, are linked via O-glycosidic linkages to sugar residues. Recent studies performed in our laboratory have led to the molecular characterization of a novel MUC1 repeat array minus mRNA, generated by an alternative splicing event that deletes the central tandem repeat array and its flanking sequences. The conceptually derived amino acid sequence of the novel MUC1 protein shows that it is identical with the previously reported transmembrane MUC1 amino acid sequence except for the deletion of the central 20 amino acid tandem repeat array and sequences immediately flanking the repeat array. This indicates that the novel MUC1 protein, which is devoid of the "hallmark" feature of mucins, the tandem repeat array, may be functionally different to the much larger, heavily glycosylated polymorphic repeat array containing MUC1 proteins, that affect cell-cell interactions. Based on an analysis of its peptide sequence, we propose the hypothesis that the novel MUC1 protein may act as a receptor molecule that modulates signal transduction. Preliminary experimental data supports this hypothesis. It appears, therefore, that the MUC1 gene is multifunctional with regard to its protein products- the repeat array containing MUC1 proteins may alter cellular adhesion processes whereas the novel MUC1 protein could be acting as a receptor-like molecule participating in signal transmission.

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N-acetylgalactosamine glycosylation of MUC1 tandem repeat peptides by pancreatic tumor cell extracts.

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Nishimori I, Perini F, Mountjoy KP, Sanderson SD, Johnson N, Cerny RL, Gross ML, Fontenot JD, Hollingsworth MA.

Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha 68198-6805.

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Synthetic peptides corresponding to the human mucin MUC1 tandem repeat domain (20 residues) were glycosylated in vitro by using UDP-N-[3H]acetyl-D-galactosamine (GalNAc) and lysates of pancreatic tumor cell lines. Results obtained with peptides of different lengths (from one to five repeats) suggest that increasing the number of tandem repeats has neither a positive nor a negative effect on the density of glycosylation along the MUC1 tandem repeat protein backbone. Purified glycopeptides were sequenced on a gas-phase sequencer, and glycosylated positions were determined by measuring the incorporated radioactivity in fractions collected following each round of Edman degradation. The results showed that two of three threonine residues on the MUC1 tandem repeat peptides were glycosylated by pancreatic tumor cell lysates at the following positions: GVTSAPDTRPAPGSTAPPAH (underlined T indicates position of GalNAc attachment). None of the serine residues were glycosylated. Determination of the mass of the glycopeptides by mass spectrometry confirmed that a maximum of two molecules of GalNAc were covalently linked to each 20-residue repeat unit in the peptides. The data presented here show that acceptor substrate specificity of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase detected in lysates of pancreatic and breast tumor cell lines is identical and is limited to some but not all threonines in the MUC1 tandem repeat peptide sequence. The influence of primary amino acid sequence on acceptor substrate activity was evaluated by using several peptides that contain single or double amino acid substitutions (relative to the native human MUC1 sequence). These included substitutions in the residues that were glycosylated and substitutions of the surrounding primary amino acid sequence. The results of these studies suggest that primary amino acid sequence, length, and relative position of the residue to be glycosylated dramatically affect the ability of peptides to serve as acceptor substrates for the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase.



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